

ANTICONVULSANT ACTIVITY OF *MORINGA OLEIFERA* LEAF

Amrutia Jay N*, Lala Minaxi, Srinivasa U., Shabaraya A. R., Moses Rajan Samuel
Srinivas college of Pharmacy, Valachil, Mangalore, Karnataka, India

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*Amrutia Jay N, Srinivas College of Pharmacy, Valachil, Mangalore-574143, India, Email: jayretent@gmail.com

ABSTRACT

Moringa oleifera which is commonly known as drumstick tree has been used for its nutrition value and extensively used as a CNS depressant traditionally. Present work has been carried out to evaluate the anticonvulsant activity of methanolic extract of *M. oleifera* leaves against pentylenetetrazole (PTZ) and maximal electroshock (MES) induced convulsions at different dose level (200 mg/kg and 400 mg/kg i.p.). Diazepam and phenytoin (5mg/kg i.p. and 25mg/kg i.p., respectively) were used as a reference standard. At both the doses it significantly ($P < 0.0001$) delayed the onset of clonic seizures in PTZ induced convulsions and significantly reduced ($P < 0.0001$) duration of hind limb extension in MES test. The phytochemical investigation of plant revealed the presence of alkaloids, flavonoids, tannins and saponins as major constituents. The data obtained indicates that methanolic extract of *M. oleifera* leaves may help to control grand mal and petit mal epilepsy.

KEYWORDS *Moringa oleifera* leaves, anticonvulsant activity, pentylenetetrazole, maximal electroshock.

INTRODUCTION

Moringa oleifera (Moringaceae) commonly known as 'Sobhanjana' is a graceful tree with corky grey bark and easily breakable branches. It grows at a height of 25 to 30 feet and it is widely grown in Sub-Himalayan range and commonly cultivated in India and Burma. Leaves usually are tripinnate, rachis slender, thickened and articulated at the base; leaflets elliptic or obovate, rounded at the apex, nerves obscure. Flowers white in large puberulous axillary panicles. The bark, root, fruit, flowers, leaves, seeds and gum of this plant are traditionally used as antispasmodic, stimulant, expectorant and diuretic. The plant is a cardiac and circulatory tonic and antiseptic. In Ayurveda and Siddha, leaves and root are used as anthelmintic, nausea, giddiness, tuberculosis etc^{1, 2}. It also has been shown to exhibit anti-inflammatory³, wound healing⁴, antioxidant⁴, analgesic activities. No scientific reports were found describing *Moringa oleifera* leaves anticonvulsant activity in our literature study. PTZ induced and maximal electroshock induced convulsions are the two primary bioassays employed in the in vivo screening of new anticonvulsant compounds^{5, 6}. So present study was carried out to evaluate anticonvulsant activity of *Moringa oleifera* leaves extract by using PTZ induced and maximal electroshock induced seizures.

MATERIALS AND METHOD

Collection of plant material and extraction

Moringa oleifera leaves were collected from local areas of Mangalore, Karnataka and authenticated by Dr. Ummanabad Srinivasa, Dept. of Pharmacognosy, Srinivas College of Pharmacy, Mangalore. Specimen has been submitted to dept. of Pharmacognosy of Srinivas College of pharmacy with reference number USMO-018 / 2011.

The leaves were shade dried and crushed. 100 gram of leaves was extracted with 90% of methanol in a soxhlet apparatus for 24 hours. Greenish extract was then evaporated under water bath to get thick mass and then air dried and kept in desiccator until further use. Yield obtained was 8% w/w.

Animals

Adult male albino mice (30±4 g) were maintained in a controlled temperature (22±2°C) with a 12h light/dark cycle and food and water ad libitum. The institutional animal ethics committee (IAEC) approved the protocol with reference no. SCP/CPCSEA/P06/F150/2011

Drugs

Pentylenetetrazol (Hi MEDIA), Diazepam (Calmpose inj. Ranbaxy, India) and phenytoin injection (Ranbaxy) were used as standard for experiment.

Phytochemical screening

Preliminary phytochemical tests were carried out for the detection of the chemical constituents like carbohydrates,

tannins, saponins, alkaloids, glycosides and flavonoids as per the standard procedure⁷.

Assessment of Anticonvulsant activity

Maximal electroshock induced seizures (MES)

MES model was used to evaluate the anticonvulsant activity of the extract.⁸ The electrical stimulus (50 mA; 60 Hz; 0.2-s duration) was applied through ear-clip electrodes using an electroconvulsimeter. Animals were grouped into four (n=6). Two groups were treated with test doses (200 mg/kg and 400mg/kg, i.p.), and one group was treated with standard Phenytoin (25 mg/kg, i.p.) and last was kept as a control. Electroshock was given by ear electrodes 30 minutes after the administration of standard drug and plant extract. Here decreased duration of Hind Limb Tonic Extension (HLTE) and mortality was considered as a protective measure against MES induced seizures.

Pentylentetrazole (PTZ) induced seizures

Animals were grouped into four (n=6). Two groups were treated with test doses (200 mg/kg and 400mg/kg, i.p.), and one group was treated with standard Diazepam (5 mg/kg) and last was kept as a control. After 30 minutes, PTZ was administered at a dose of 80 mg/kg i.p. and observed for convulsive behaviour for 30 mins. The parameters like onset of myoclonic jerks, clonus, tonic extension and mortality were measured for anticonvulsant property⁹.

Statistical analysis

All the values were expressed as arithmetic mean \pm SEM and were analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's test. P value less than 0.05 (P<0.05) was the critical criterion for statistical significance.

RESULT

Maximal electroshock test

The extract showed significant decrease in the duration of hind limb tonic extension (HLTE) induced by maximal electroshock (P<0.0001) but was unable to completely prevent its occurrence. The effect of methanolic extract of *Moringa Oleifera* was in dose dependent manner. Diazepam completely protected mice against maximal electroshock induced convulsion. 100% protection was seen at dose level 400 mg/kg.

Pentylentetrazole induced seizures

The methanolic extract of *M. oleifera* and diazepam significantly prevented PTZ induced seizures. The effect of methanolic extract of *M. oleifera* was seen in dose dependent manner. It showed significant (P<0.0001) delayed in onset of jerks and clonic convulsion. Moreover 100% protections were seen in both the doses.

DISCUSSION

Methanolic extract of *Moringa oleifera* leaves inhibited MES and PTZ induced seizures. These are the two most common and predictive tests for screening of the anticonvulsant activity. Anticonvulsant activity in PTZ-induced seizures identifies compounds that can raise seizure threshold in brain¹⁰. Anti-epileptic drugs effective in the therapy of generalized seizures of petit mal type (absence of myoclonic) i.e. phenobarbitone, valproate, ethosuximide and benzodiazepines suppress PTZ-induced seizures in a dose-dependent manner¹¹. Anticonvulsant activity against PTZ induced seizures also identifies compounds that can raise seizure threshold in brain^{12, 13}. Antagonism of PTZ induced seizures also indicates that methanolic extract has interaction with GABAergic neurotransmission as PTZ interact with GABA receptor, specifically GABA_A receptor¹⁴. Moreover, previous studies have shown that flavonoids may cause facilitation of GABAergic system¹⁵. Preliminary phytochemical study showed the presence of flavonoids in the Methanolic extract of *M. oleifera* leaves.

The methanolic extract was not able to abolish HLTE at all the doses used in this study but significantly reduced its duration. HLTE is the universal feature of maximal electroshock in mice, rats, rabbits, cats, monkeys and humans¹⁰. Abolition of HLTE in MES test predicts the ability of drug to prevent the spread of seizure discharge from the epileptic focus and its effectiveness in MES test correlates well in suppressing generalized tonic-clonic seizures^{16,17}. All the currently available antiepileptic drugs that are used to treat generalized tonic-clonic seizures (e.g. Phenytoin, lamotrigine, and carbamazepine) are effective in the MES test¹⁸. From the significant activity shown by the Methanolic extract of *M. oleifera* in the MES test, it may be concluded that it is effective against the generalized tonic-clonic convulsion.

CONCLUSION

It was concluded that the methanolic extract of *M. oleifera* leaves possess anticonvulsant properties. Further study is required for isolation and identification of active constituents and to confirm exact mechanism.

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REFERENCES

1. Nadkarni K.M: Indian Materia Medica, Popular Prakashan, Mumbai, India, 3rd Ed. 1995, Pg-813.
2. Oommen S, Ved DK and Krishnan R: Tropical Indian Medicinal plants, FRLHT, Bangalore.2000:245

3. Chandrashekar K.S and Thakur A: Antiinflammatory activity of Moringa oleifera stem bark extracts against Carrageenan induced rat paw oedema, J.Chem.Pharm.Res. 2010;2(3):179.
4. Charde RM and Charde MS: Evaluation of ethanol extract of Moringa oleifera for Wound healing, anti-inflammatory and antioxidant activities on rats, Hamdard Medicus 2009;52(2):59-67.
5. Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA. Antiepileptic drug development: II. Anticonvulsant drug screening. *Epilepsia*. 1978;19(4):409-428.
6. Loscher W, Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. *Epilepsy Res.* 1988;2(3):145-181.
7. Dr. Kokate CK. Practical Pharmacognosy 4th edition, Vallabh Prakashan, 2005:108-111.
8. Manocha A, Sharma KK, and Mediratta PK. Possible mechanism of anticonvulsant effects of ketamine in mice, *Indian J. Exp. Biol.* 2001; 39:1002-1008.
9. Mahmoodi M, Zohoor A and Asadi M. Anticonvulsant effect of sour orange flowers extract in experimental pentylenetetrazol-induced seizures in rat. *Arch Inn Med.* 2003; 6(3):212-213.
10. Raza M, Shaheen F, Choudhary MI, Sombati S, Rafiq A, Suria A, Rahman A, DeLorenzo RJ. Anticonvulsant activities of ethanolic extract and aqueous fraction isolated from *Delphinium denudatum*. *J Ethnopharmacol.* 2001;78(1):73-78.
11. Loscher W, Fassbender CP, Nolting B. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models. *Epilepsy Res.* 1991;8(2):79-94.
12. Goodman LS, Grewal MS, Brown WC and Swinyard BA. Comparison of maximal seizures evoked by pentylenetetrazol (metrazol) and electroshock in mice and their modification by anticonvulsants. *J. Pharmacol. Exp. Ther.* 1953; 108:168-176.
13. Piredda SG, Woodhead JH and Swinyard EA. Effect of stimulus intensity on the profile of anticonvulsant activity of phenytoin, ethosuximide and valproate. *J. Pharmacol. Exp. Ther.* 1985; 232:741-745.
14. De Sarro A, Cecchetti V, Fravolini V, Naccari F, Tabarrini O and De Sarro G. Effects of novel 6-desfluoroquinolones and classic quinolones on pentylenetetrazole-induced seizures in mice. *Antimicrob. Agents Chemother.* 1999; 43:1729-1736.
15. Fernandez SP, Wasowski C, Loscalzo LM, Granger RE, Gohnston GA, Paladini AC, et al. central nervous system depressant action of flavonoid glycosides. *Eur J Pharmacol.* 2006; 539:168-76.
16. Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA. Antiepileptic drug development: II. Anticonvulsant drug screening. *Epilepsia*. 1978; 19(4):409-428.
17. Porter RJ, Cereghino JJ, Gladding GD, Hessie BJ, Kupferberg HJ, Scoville B, White BG. Antiepileptic Drug Development Program. *Cleve Clin Q.* 1984; 51(2):293-305.
18. Castel-Branco MM, Alves GL, Figueiredo LV, Falcao AC and Caramom MM. 2009. The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. *Methods Find. Exp. Clin. Pharmacol.* 2009;31:101-116.

Table 1: Effect of methanolic extract of *M. oleifera* leaves against MES induced convulsions

Group	Treatment	Dose	Duration of limb extensor phase(sec)	% Mortality
1	Control	-	17.30±0.49	0
2	Phenytoin	25 mg/kg	-	100
3	MEMO	200 mg/kg	14.37±0.58***	83.33
4	MEMO	400 mg/kg	8.85±0.36***	100

MEMO= Methanolic extract of *Moringa oleifera*, All the values are expressed as mean ±SEM one way ANOVA followed by Dunnett's 't' test. n=6, ***P<0.0001

Table 2: Effect of methanolic extract of *M. oleifera* against PTZ induced convulsions

Group	Treatment	Dose	Onset of		Tonic (sec)	% Mortality
			Jerks(sec)	Clonic(sec)		
1	Control	-	43.00±4.98	63.17±5.34	421.7±5.50	0
2	DZP	5 mg/kg	-	-	-	100
3	MEMO	200 mg/kg	79.17±6.03***	112.5±7.23***	-	100
4	MEMO	400 mg/kg	98.17±4.95***	141.0±5.72***	-	100

MEMO= methanolic extract of *Moringa oleifera*, All the values are expressed as mean ±SEM one way ANOVA followed by Dunnett's 't' test. n=6, ***P<0.0001

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